cies (Bouix-Busson et al., 1984; Vareille-Morel et al., 1994). The shell height of 2 mm must be considered, in the case of *L. fuscus,* as the maximum size in which the snail might sustain the full larval development of *F. hepatica.*

The small number of metacercariae found in the 1-mm *L. fuscus* (21.4/snail) fell within the range of figures reported by Busson et al. (1982) in another species of *Lymnaea* when they are exposed to *F. hepatica* miracidia during their first days of life (from 10 to 35 metacercariae per infected snail). Cercarial production was thus limited in the case of *Lymnaea* snails infected in the first days following their hatching, and this process seemed to be independent of snail species.

As most of the other *Lymnaea* species that lived in western Europe, *L. fuscus* might be classified in the list of snails showing a partial resistance to the larval development of *F. hepatica* (Boray, 1978), due to snail age. Only young snails of 2 mm or less were able to sustain full development of the parasite. An explanation may be related to the report by Dikkeboom et al. (1984) for *Lymnaea stagnalis.* According to these authors, the defense system of juvenile snails does not mature until the end of their first week of life. In the *Lymnaea* species of western Europe other than *L. truncatula,* it is hypothesized that the immaturity of the defense system in newborns and 1-mm young snails would permit the larval development of *F. hepatica* only in these snails.

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Isolation of *Sarcocystis speeri* Dubey and Lindsay, 1999 Parasite from the South American Opossum (*Didelphis albiventris*) from Argentina

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ABSTRACT: *Sarcocystis* sporocysts from the intestines of 2 opossums (*Didelphis albiventris*) from Argentina were fed to gamma-interferon knockout (KO) and nude mice. Protozoal schizonts were seen in brain, liver, spleen, and adrenal glands of mice examined 33–64 days after feeding sporocysts. Sarcocysts were seen in skeletal muscles of KO mice 34–71 days after feeding sporocysts. Schizonts and sarcocysts were structurally similar to *Sarcocystis speeri* Dubey and Lindsay, 1999 seen in mice fed sporocysts from the North American opossum *Didelphis virginiana* from the United States.

The North American opossum (*Didelphis virginiana*) is the definitive host for at least 3 pathogenic species of *Sarcocystis*: *Sarcocystis falcatula* (Box and Duszynski, 1978; Box et al., 1984), *Sarcocystis neurona* (Fenger et al., 1997; Dubey and Lindsay, 1998; Dubey and Lindsay, 1999), and *Sarcocystis speeri* (Dubey, Speer, and Lindsay, 1998; Dubey and Lindsay, 1999). These species of *Sarcocystis,* transmitted via opossum feces, have been reported only from the United States. The sporocysts of these 3 species are similar morphologically but can be distinguished by their pathogenicity and infectivity to birds and immunodeficient mice, e.g., *S. falcatula* is not infective to mice, whereas *S. neurona* and *Sarcocystis speeri* are not

infective to birds (Marsh et al., 1997; Dubey and Lindsay, 1998; Dubey et al., 1998; Dubey and Lindsay, 1999). Both *S. neurona* and *Sarcocystis speeri* can induce encephalitis in mice associated with schizonts and merozoites (Dubey et al., 1998; Dubey and Lindsay, 1999). Sarcocysts of *S. neurona* have not been detected to date. Sarcocysts were seen in γ-interferon knockout (KO) mice fed sporocysts of *Sarcocystis* sp. (Dubey et al., 1998). We report another host, *Didelphis albiventris* from Argentina as a definitive host for *Sarcocystis speeri* (Dubey and Lindsay, 1999).

Sarcocystis sporocysts were obtained from 2 adult male opossums (*D. albiventris*) trapped on a farm in Chascomús, located 80 km from La Plata in Buenos Aires Province, Argentina. The epithelium was scraped from the small intestine of the opossums, homogenized in a blender and digested in 1% aqueous sodium hypochlorite for 10 min. After repeated centrifugations to remove chlorine, the homogenate was filtered through a 100-μm-mesh sieve, and the sporocysts collected were suspended in an aqueous solution containing 100 μg of streptomycin and 200 units of penicillin for transport by air to the Beltsville Agricultural Research Center (BARC). At Beltsville, the sporocysts were suspended in antibiotic saline containing 10 mg streptomycin, 10,000 units penicillin, 500 units mycostatin, and 0.05 mg fungizone per ml of Hank's balanced salt solution (Leek and Fayer, 1979). Sporocysts were counted in a hemacytometer and stored at 4 C.

Sporocysts were fed to 15 KO mice (BALB/c-Ifng^{tm1Ts}) and 6 nude mice (C57BL/6JHFH11-Nu) obtained from Jackson Laboratories (Bar Harbor, Maine)(Table I). Mice that were killed or died were necropsied. Portions of all internal organs, eyes, and skin were fixed in 10% buffered neutral formalin and processed for histology. Paraffin-embedded sections were cut at 5 μm and examined after staining with hematoxylin and eosin. For immunohistochemical staining, paraffin sections were reacted with anti-*S. neurona* antibody using techniques and reagents described previously (Lindsay and Dubey, 1989; Hamir et al., 1993), except that sections were treated with 0.4% pepsin and anti-*S. neurona* antibody was diluted 1:1,000. Antibodies to *S. neurona* had been obtained from rabbits immunized with the SN2 isolate of *S. neurona* isolated from a paralyzed horse (Hamir et al., 1993).

Tissues from a mouse experimentally infected with *S. neurona* (Dubey and Lindsay, 1998) and from a naturally infected horse (Dubey et al., 1991) were used as positive controls for *S. neurona* infection.

For the attempted transmission of *Sarcocystis* species from the Argentinian opossum, 2 Swiss Webster 25-g female mice (Taconic Farms, Germantown, New York) and 3 C57BL/6 female 20-g mice (Jackson Laboratories, Maine) were each fed sporocysts from opossum 2 (the same dilution as fed to KO mice 4305 to 4307, see Table I). The mice were killed 61 days after feeding sporocysts, and their tissues were examined histologically for *Sarcocystis.* In addition, carcasses of these mice were homogenized in a blender, digested in acid pepsin solution for 1 hr at 37 C, and the digest examined microscopically for *Sarcocystis* bradyzoites (Dubey et al., 1989).

Leg muscles from the KO mouse 4216 that died 48 days and mouse 4218 killed 64 days after feeding sporocysts (DAFS)(Table I), were fixed in Karnovsky's fixative and prepared for electron microscopy as described (Speer and Dubey, 1998).

Asexual stages of *Sarcocystis* sp. were found in tissues of mice (Figs. 1–5) fed sporocysts from both opossums from Argentina. Protozoa were not seen in tissues of the 2 KO mice killed 10 and 11 DAFS (Table I). A few merozoites were seen in sections of the liver of the mouse killed 12 DAFS. Schizonts were seen in brain, spleen, liver, and adrenal gland. Sarcocysts were seen in skeletal muscles of the carcass, tongue, and ocular muscles, but not in the heart. Sarcocysts were occasionally associated with severe myositis in KO mice. In the brain, schizonts were associated with nonsuppurative encephalitis. Tissues from mice did not react with anti-*S. neurona* antibody.

Schizonts observed in tissues of KO or nude mice were structurally similar to those of *Sarcocystis* sp. (Dubey et al., 1998). Sarcocysts were filiform and up to 5 mm long. The sarcocyst wall was thin and had spikelike projections under the light microscope. By transmission electron microscopy the structure of the sarcocyst wall (Fig. 6) was the same as described by Dubey et al. (1998).

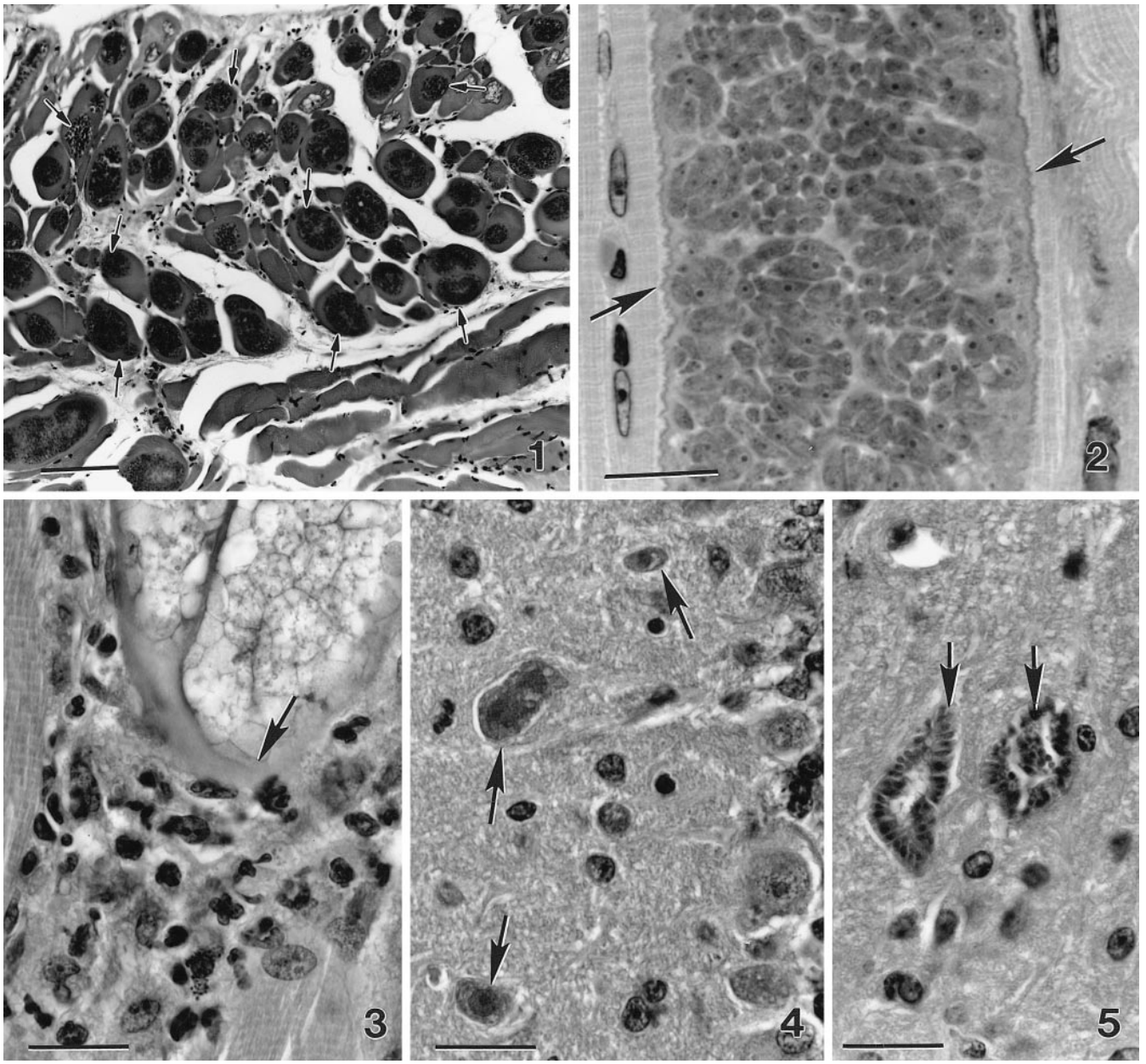
Sarcocystis bradyzoites were not seen in muscle digests of outbred mice fed sporocysts from opossum 2, suggesting that this species of *Sarcocystis* is probably not transmissible to outbred mice. The number of sporocysts of *Sarcocystis* sp. was not determined because both opossums also had *S. falcatula* sporocysts (Dubey et al., 1999).

Sarcocystis neurona-induced equine protozoal encephalo-

TABLE I. Sarcocystosis in mice fed sporocysts from opossums from Argentina.*

Inoculum opossum no.	Mouse type	Day killed or died*	Stages of *Sarcocystis*	
			Schizonts	Sarcocysts
1	KO 4218	64	NS	Muscle, tongue
	KO 4217	71	NS	Muscle, tongue, ocular muscles
2	KO 4229	10	NS	NS
	KO 4227	11	NS	NS
	KO 4215	12	Liver, spleen	NS
	KO 4306	34	NS	Muscle
	KO 4325	38	Brain	Muscle, tongue
	KO 4326	41	Brain	Muscle
	KO 4307	47	Brain	Muscle
	KO 4216	48†	NS	Muscle
	KO 4304	50†	Brain	Muscle
	KO 4305	58	Brain	Muscle
	KO 4306	34	Brain	Muscle
	KO 4307	47	Brain	Muscle
	KO 4300	57	Brain	Muscle
	Nude 4308	33	Liver, spleen	NS
	Nude 4309	39†	Liver, spleen, adrenal	Muscle (few)
	Nude 4543	33	Liver, spleen	Muscle (few)
	Nude 4540	43†	Liver, spleen	NS
	Nude 4541	43†	Liver, spleen	NS
	Nude 4542	40	Liver, spleen	NS

* KO = knockout; NS = not seen.
† Died.



FIGURES 1–5. *Sarcocystis* stages in sections of tissues of γ-interferon knockout mice fed sporocysts from opossums from Argentina. Hematoxylin and eosin stain. **1.** Numerous sarcocysts (arrows) in abdominal muscle of mouse 4216, 48 days after feeding sporocysts (DAFS). Bar = 100 μm. **2.** Longitudinal section of a sarcocyst. Note thin cyst wall with spikes (arrow). Mouse 4218, 64 DAFS. Bar = 15 μm. **3.** Degenerating sarcocyst and leukocyte infiltrations at the edge of the sarcocyst (arrow). Mouse 4218, 64 DAFS. Bar = 20 μm. **4, 5.** Immature (arrows) and 1 mature (double arrows) schizonts in the brain of mouse 4218, 64 DAFS. Bar = 20 μm.

myelitis (EPM) is a serious neurologic disease of horses in North America, Brazil, and Panama (Dubey et al., 1991; MacKay, 1997). As yet EPM has not been reported from Argentina. The distribution of EPM closely parallels the range and distribution of the opossum *D. virginiana*. Whether *D. albiventris* is also a host for *S. neurona* is unknown. One way to identify the presence of *S. neurona* in opossum feces is by infectivity to KO or nude mice (Dubey et al., 1998).

Didelphis albiventris (previously known as *Didelphis azarae* and *Didelphis paraguayensis*) is distributed in the tropical and temperate zones of South America, including the Andean countries, except Chile. It is found throughout Argentina. The results of this investigation indicate that *D. albiventris* is another definitive host for *Sarcocystis* sp. and should be considered when attempting to isolate *S. neurona* sporocysts from opossums in South America.

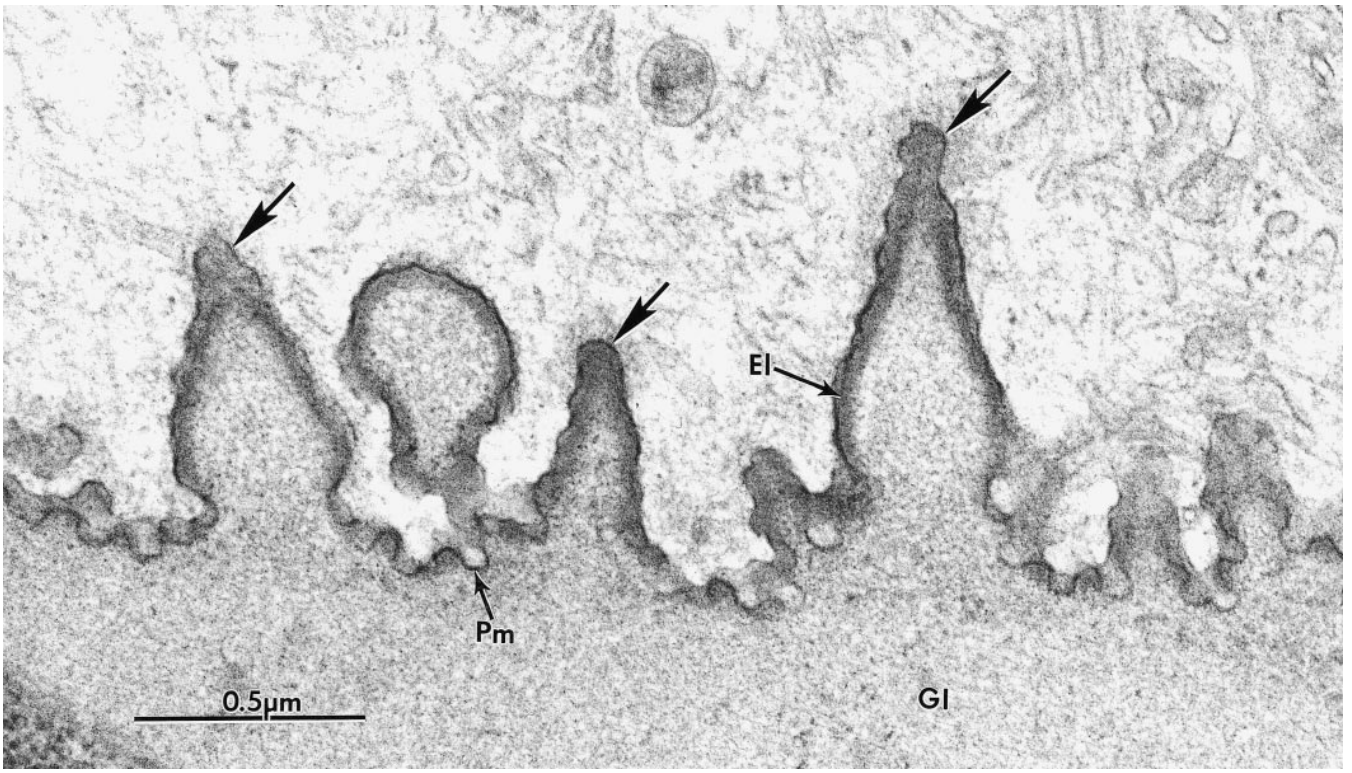


FIGURE 6. Transmission electron micrograph of the sarcocyst wall in leg muscle from KO mouse 4218, 64 days after infection. The wall consists of steeple-like projections surmounted by a spire (arrows); El, electron-dense layer; Gl, granulate layer; Pm, parasitophorous vaculoar membrane.

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